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Metabolic-vascular coupling in skeletal muscle: a potential role for capillary pericytes?

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ABSTRACT

The matching of capillary blood flow to metabolic rate of the cells within organs and tissues is a critical microvascular function which ensures appropriate delivery of hormones and nutrients, and the removal of waste products. This relationship is particularly important in tissues where local metabolism, and hence capillary blood flow, must be regulated to avoid a mismatch between nutrient demand and supply that would compromise normal function. The consequences of a mismatch in microvascular blood flow and metabolism are acutely apparent in the brain and heart, where a sudden cessation of blood flow, for example following an embolism, acutely manifests as stroke or myocardial infarction. Even in more resilient tissues such as skeletal muscle, a short-term mismatch reduces muscle performance and exercise tolerance, and can cause intermittent claudication. In the longer-term, a microvascular-metabolic mismatch in skeletal muscle reduces insulin-mediated muscle glucose uptake, leading to disturbances in whole body metabolic homeostasis. While the notion that capillary blood flow is fine tuned to meet cellular metabolism is well-accepted, the mechanisms that control this function and where and how different parts of the vascular tree contribute to capillary blood flow regulation remains poorly understood. Here, we discuss the emerging evidence implicating pericytes, mural cells that surround capillaries, as key mediators that match tissue metabolic demand with adequate capillary blood flow in a number of organs, including skeletal muscle.

KEY WORDS: Pericytes, capillary blood flow, microvasculature, skeletal muscle

1. INTRODUCTION

1.1 Regulation of blood flow

From a top-down point of view, whole body blood flow is equal to the total cardiac output. At the organ level, cardiac output is rationed between organs to meet their metabolic demands and specific functions. As an example, the brain receives a relatively constant proportion of total cardiac output (15-20%) to ensure appropriate supply of nutrients to working neurons (1). The kidneys receive approximately the same amount of blood flow to ensure adequate blood filtration and blood volume/pressure homeostasis (1). In general, total organ blood flow, is determined by the diameter of the arteriolar resistance vessels within a specific organ. The appropriate rationing of cardiac output between organs has long been proposed to be a balance between two opposing factors: (i) the sympathetic nervous system (SNS) mediating vasoconstriction to maintain mean arterial blood pressure; and (ii) organ-specific metabolic rate-mediated vasodilation and increased blood flow to ensure appropriate cellular/tissue function (2). While it is well accepted that the SNS innervates arteries and arterioles to regulate their tone and hence blood flow, it remains to be determined how increased tissue metabolism can stimulate site-specific increases in local capillary blood flow.

1.2 Regulation of local blood flow

Within almost all organs, local tissue metabolic demand can vary at any given time. This is best exemplified by skeletal muscles where resting metabolism is low and hence capillary perfusion is appropriately limited to conserve whole body metabolic homeostasis. In contrast, during intense exercise, metabolic demand within contracting muscles can increase as much as 100-fold and this is matched by equal increases in capillary perfusion (2). Ever since the work of Nobel Prize winning physiologist August Krogh in the 1920s, the prevailing view has emphasized arterioles as regulators of capillary blood flow and therefore determinants of muscle nutrient supply (3). While arterioles undoubtedly play an integral role in this process, for appropriate coupling of metabolism and blood flow, the capillary network itself must be able to detect and respond to local tissue derived signals to

appropriately regulate both capillary and arteriolar perfusion. Despite the early documentation by Krogh, the physiological and biochemical mechanisms that dictate this local metabolic-vascular coupling remain to be identified and characterized.

1.3 Circulating versus paracrine factors in the regulation of local blood flow

From a systemic view point, circulating factors such as angiotensin II modulate arterial tone to regulate and maintain mean arterial pressure. Importantly, angiotensin II, as well as other circulating factors such as insulin, can also have profound and specific effects within the skeletal muscle vasculature to regulate perfusion and hence muscle metabolism (4). In contrast, metabolites (e.g. adenosine) and ions (e.g. K^+) derived from muscle metabolism are released into the extracellular space and are proposed to mediate a prompt and highly localised vascular response to increase capillary blood flow in a site-specific manner via a mechanism termed ascending vasodilation (5, 6). Championed by Steven Segal and colleagues (5-7), this involves the initiation and transmission of a hyperpolarizing wave in a cell-cell manner via gap junctions upstream to feed arteries to increase arteriolar blood flow and hence capillary perfusion (see Bagher and Segal 2011 for review) (6). However, much of what is known about these vascular integrating mechanisms was derived from local application of acetylcholine (ACh), which may activate different signaling pathways than the signals derived from muscle metabolism. Following up on these studies, others have utilised single muscle fibre stimulation to show that these conducted vasodilatory signals can originate in capillaries and propagate via gap junctions to upstream arterioles to modulate blood flow (8-10). While some evidence suggests that purinergic signaling within the muscle capillary network may mediate this sort of response to increasing metabolism (11), how and where this is sensed, integrated and transmitted through the microvascular network remains to be fully understood. Intriguingly, while the presence of pericytes around capillaries in skeletal muscle has been documented for over 40 years (12), to our knowledge only two recent studies have reported that skeletal muscle pericytes can actively modulate capillary diameter. The first utilised intravital microscopy of the cremaster muscle microcirculation to demonstrate pericyte responsiveness to

endothelin-1 (13). The second study utilised two-photon microscopy to demonstrate that hyperemia-induced changes in capillary diameter in muscle occurred preferentially in pericyte-covered capillary segments and that this vasodilator response was reduced in states of pericyte depletion (14). Based on these emerging evidence from muscle and the ability of pericytes to regulate capillary perfusion in response to increased metabolism in other organs (e.g. the brain) (15), pericytes are ideally positioned between capillaries and skeletal myofibres to be able to sense the metabolic needs of muscle cells and appropriately regulate capillary blood flow.

2. Pericytes and capillary blood flow

The term pericyte was first coined in 1923 (16) and is now used to describe a type of mural cell within the capillary basement membrane. Pericytes encircle capillaries through the extension of cytoplasmic processes that span numerous endothelial cells and often multiple adjacent capillaries (see figure 1); a morphological feature with poorly understood physiological function (17). Of note, pericytes share a number of common features with vascular smooth muscle cells (VSMC) (18) including the cellular machinery necessary for constriction and dilation of blood vessels (19, 20). Pericytes are reported to have a number of functions and can behave as multipotent progenitor cells to facilitate tissue repair including in muscle (21-23). Pericytes have also been reported to actively support angiogenesis (24-26) and capillary function (27) through the release of vascular endothelial growth factor (28). Importantly, brain and retinal pericytes have been shown to contract or relax in response to a number of circulating factors, including angiotensin II and insulin (29, 30), as well as relax in response to cell-metabolism derived factors such as adenosine, lactate and carbon dioxide (CO_2) (31-33). In the brain, pericytes form an interconnected network within the microvasculature and are capable of transmitting signals upstream to activate adjacent pericytes (34). A recent study calculated that the speed of these conducted responses ranges from 5-20µm/s and that the signal was transmitted both up and downstream of the initiating pericyte (35). This rapid cell-cell communication is particularly intriguing and suggests that rather than acting independently on single capillaries, pericytes may regulate and co-ordinate networks of capillaries through an

integrated and interconnected response to meet tissue metabolic demand. Indeed, we have unpublished histological evidence that this may be the case within the skeletal muscle capitally network (see figure 1). Given their similarity to VSMC's (18), it is also plausible that pericytes may be able to transmit this same signal upstream to dilate or constrict precapillary arterioles to appropriately regulate capillary perfusion. Based on their position at the interface between the capillaries and the cells those capillaries are supplying, pericytes are ideal candidates for the integration of both circulating and local factors to effectively couple blood flow to local metabolism.

2.1 Pericytes and capillary blood flow regulation in the brain

Most of what we know about pericytes stems from studies investigating their role in the brain microcirculation. Indeed, it has been proposed that among all microvascular beds, the brain contains the greatest density of capillary-bound pericytes (36). The intimate association of pericytes with the basement membrane, and complex series of tight junctions and channels linking pericytes and adjacent cells, including endothelial cells, makes their role integral to the structure and function of the blood-brain barrier (37-42). From the abluminal, tissue side, neurons and astrocytes closely associate with brain capillaries at sites immediately adjacent to pericytes (41, 42). This specific co-localisation of cells within the brain microvasculature, termed the neurovascular unit, is the location where nutrient needs of neurons are sensed and integrated into the microvasculature to effect changes in local capillary perfusion; a process called neurovascular coupling. Here, neurotransmitters (e.g. glutamate) and ions (e.g. K^+) can be released into the perivascular space where they are sensed by pericytes and endothelial cells to regulate capillary tone and perfusion (37). During periods of increased neural activity, capillary diameter where pericytes reside increases prior to any changes at upstream pre-capillary arterioles (43-45). In response to local glutamate release, pericytes relax and increase capillary diameter through a nitric oxide-dependent activation of the prostaglandin E_2 receptor (15). These were some of the first studies to demonstrate that pericytes are capable of directly affecting capillary diameter and hence blood flow in the brain. Further evidence has recently come from studies investigating the roles that pericytes play in

regulating capillary perfusion in the context of ischemic stroke (15) and Alzheimer's disease (46). Within these pathological settings, pericytes appear to be highly sensitive to ischemia and amyloid β and both cause pericyte constriction that limits capillary blood flow independently of changes in arteriolar tone. Taken together, the above data provide strong evidence for a functional role of pericytes in the tight regulation of capillary blood flow in the brain and their dysregulation in disease states.

2.2 Pericytes and capillary blood flow regulation in the heart

The coronary microcirculation is critical for the normal functioning of the heart, and as with the brain, is acutely affected during periods of ischemia. Despite being the second most abundant cell type in the heart after endothelial cells (47), pericytes retain similar roles as in the brain, including regulation of angiogenesis, barrier potential and selectivity and regulation of capillary blood flow (47). As in the brain, constriction of pericytes has been implicated in the context of myocardial infarction where ischemia causes a powerful constriction to occlude capillaries, which intriguingly can be overcome using intravenous administration of adenosine (47-49). Interestingly, cardiac pericytes may also contribute to the origin of a myocardial infarction through their potential involvement in development of atherosclerosis and vessel stiffening (49). Via a series of pericyte-endothelial cell and pericyte-pericyte gap junctions, cardiac pericytes have been described as forming a functional syncytium capable of propagating electrical and metabolic signals along the vascular tree, akin to the cardiomyocytes themselves (47). Pericytes have also been located within the intima of arterioles and venules of the coronary circulation, appearing to be far more widely distributed along the angioarchitecture with respect to those found in other organs (47). These observations within the heart further implicate the potential role of pericytes as integrators and propagators of metabolic signals where they may not only act locally and directly on capillaries but also signal further upstream to arterioles to modulate total blood flow.

2.3 Pericytes and capillary blood flow regulation in the retina

Due to its developmental origins, the retina is considered a part of the central nervous system (50) and as such the vasculature has similar characteristics to that in the brain. The idea that capillary perfusion is intrinsically linked to photoreceptor activity was first discussed in the 19th century (51). Since then, a substantial body of evidence has emerged to suggest that overall increases in total retinal flow (52-54), as well as increases in microvascular flow occur in regions local to photo stimuli (55, 56). Based on pericyte function in the brain (15), investigators have inferred that pericytes may actively regulate retinal capillary blood flow. In contrast, recent experiments performed in the retina by Kornfield and Newman suggest that arterioles, not pericytes are responsible for retinal blood flow regulation in health (57). Despite this, retinal pericyte changes in conjunction with disease have been well documented, particularly in the context of diabetic retinopathy. Retinopathy is a major diabetic complication that leads to vision impairment in 35% of diabetes sufferers world-wide (58) and is characterized by the formation of microaneurysms (59, 60), increased vascular permeability (61) and angiogenesis (58). Together, these microvascular pathologies contribute to hypoxia (62) and vision impairment (63). As with acute hypoxia during ischemic stroke in the brain, chronic hypoxia in the diabetic retina is associated with pericyte dysfunction and a reduced dilatory response that can be reversed by inhibiting inducible nitric oxide synthase (62). While sharing some similar functions to pericytes in the brain and heart, whether retinal pericytes play an active role in regulating retinal capillary flow remains to be definitively determined.

2.4 Pericytes and capillary blood flow regulation in the liver

Despite comprising only ~2-3% of total adult body weight, the liver receives about 25% of total cardiac output at rest (1). To accommodate this high rate of flow, the liver has a unique circulatory system consisting of afferent and efferent vessels connected by specialized exchange vessels termed sinusoids – fenestrated capillaries lacking a basement membrane (64). Compared with capillaries in other organs, fenestrated capillaries facilitate a higher exchange rate between the blood,

endothelial cells and other cells associated with the hepatic sinusoid, including pericytes. Despite these structural capillary differences, hepatic pericytes, alternatively termed Ito cells or hepatic stellate cells, have similar function and morphology to pericytes of other tissues (65, 66). Additionally, they also have a range of liver specific functions including lipid and vitamin A storage (67, 68). Importantly, it has been shown that liver pericytes actively regulate sinusoidal tone in response to locally generated molecules such as endothelin-1 and various prostaglandins (69-73). This normal pericyte response is disrupted in fatty liver disease (70), a condition that involves the accumulation of fat within the liver hepatocytes, causing them to swell and decrease the luminal diameter of surrounding sinusoids (74, 75). Hepatocyte lipid accumulation has been shown to reduce the total number of functional sinusoids (74, 75) and increase pericyte contractility, thus greatly reducing hepatic capillary perfusion (70). Given that microvascular perfusion supports and enables normal liver function, this increased pericyte contractility and hence reduced capillary perfusion is likely to further compromise liver function.

2.5 Pericytes and capillary blood flow regulation in the pancreas

Primarily an exocrine organ, the pancreas contains a small amount of endocrine tissue, only contributing 1-3% of pancreatic volume. Despite this, the endocrine portion has been well studied as it encompasses the islets of Langerhans, housing the pancreatic beta cells responsible for insulin formation and secretion. Pancreatic beta cells are intimately associated with capillaries (76) allowing for prompt assessment of blood glucose concentration and the tailored release of insulin into the blood stream to tightly regulate blood glucose concentrations. Pericytes have been found on capillaries in pancreatic islets (77-79) with evidence suggesting they are necessary for normal beta cell function (78) and regeneration (80). For example, pericytes produce components of the extracellular matrix that promote beta cell proliferation (80), a process that is hindered when pericytes are depleted. Furthermore, it is proposed that pericytes produce growth factors required for glucose-stimulated insulin exocytosis (81). In the absence of these growth factors, beta cells dedifferentiate into an immature form and lose the ability to tailor insulin secretion to blood glucose

concentration (82). Although it appears that pericytes are required for normal insulin secretion, it is still unknown whether pericytes directly communicate with beta cells or whether the majority of these changes are mediated by pericytes manipulating capillary blood flow to beta cells. A recent publication by Almaca et al. demonstrated that pericytes can regulate capillary diameter and blood flow to islets in response to factors such as endothelin-1 (83). The same study went on to show that an increase in beta cell activity resulted in a decrease in pericyte activity, manifesting as capillary dilation (83). Furthermore, increasing concentrations of glucose decreased cytosolic calcium levels in pericytes and simultaneously dilated capillaries (83). This study also highlighted that pericyte coverage of islet capillaries decreases with type 2 diabetes (83). Given that pericytes are necessary for both normal beta cell function and normal islet capillary perfusion, it is plausible that this loss of pericytes on islet capillaries in type 2 diabetes impairs the ability of islet capillary blood flow to be coupled with the metabolic needs of beta cells. Overall, it could be hypothesized that this may compromise both glucose delivery to the beta cells and the subsequent insulin release into the blood stream, thus contributing to progression of type 2 diabetes.

3. Can pericytes regulate capillary blood flow in skeletal muscle?

Due to their substantial contribution to body mass (~40%) and need for energy to drive our movement, mobility and even breathing, skeletal muscles are important determinants of whole-body metabolism. Skeletal muscle resistance vessels supply myofibers via an intricate branching network of arterioles that give rise to terminal pre-capillary arterioles (see figure 1). Each terminal arteriole supplies a group of 15–20 capillaries, collectively known as a microvascular or capillary unit (84). Due to the low metabolic needs of muscle at rest, it is important to note not all parts of muscle capillary networks may be fully perfused at rest (85, 86). Indeed, a number of studies have shown that microvascular perfusion in resting skeletal muscle is neither continuous nor uniform, but rather intermittent and heterogeneous in distribution (87-89). Thus, it has been suggested that microvessels in muscle show rhythmic variations in diameter (vasomotion) and flowmotion to alternate blood flow through different capillary units as needed to maintain resting metabolism (86).

When metabolism increases, for example during muscle contraction, dilation of the vasculature enhances the delivery of oxygen and nutrients to active muscle fibres in concert with removing metabolic waste products (90), thereby coupling local perfusion to metabolic demand. The extent to which capillary blood flow increases during muscle contraction is related intimately to the power and force of contraction (and hence metabolic demand) (91). Importantly, blood flow is increased only to active muscles and can be specifically directed only to active fibres within a muscle (92, 93). This is reminiscent of the blood flow response in the brain (as discussed above) and suggests that the muscle microvasculature may be able to respond to highly localised metabolic changes and demands.

Given that skeletal muscle capillary units are supplied by terminal arterioles, the majority of research thus far has focused on arteriolar responses to increased metabolic demand. Indeed, numerous of studies have shown that arterioles promptly dilate in response to exercise (85, 94, 95), metabolites such as adenosine (96, 97) and prostaglandins (98, 99). Given the evidence summarized above and the anatomical organization of the muscle vasculature, it stands to reasons that arteriolar constriction/dilation is a major determinant of vascular resistance and hence capillary perfusion in muscle during exercise (5, 10). However, given the presence of pericytes around skeletal muscle capillaries, and the emerging evidence for pericyte contraction and dilation (13, 14), capillaries (via pericyte actions) are able to alter capillary blood flow and/or blood content independently from, or in conjunction with arterioles to determine nutrient delivery to skeletal muscle fibres to control local metabolism (100). While these subtle capillary flow oscillations may not be easily distinguishable during high intensity exercise, they may be identifiable at lower work rates that do not increase bulk flow or in instances where muscle capillary perfusion is increased independent of muscle contraction (100). Whether pericytes participate in or regulate capillary perfusion in these contexts remains to be determined.

As well as facilitating contraction and movement, skeletal muscles are also a major site for insulin-mediated glucose disposal and contribute greatly to whole body metabolism. For this reason, blunted insulin-mediated muscle glucose uptake has been championed as one of the early and

primary drivers of development and progression of whole body insulin resistance (101). Within muscle, an important action of insulin is to first increase capillary blood flow (102). In doing so, insulin enhances the delivery of both glucose and insulin to muscle cells to facilitate insulin-stimulated muscle glucose uptake. While local infusion of insulin into the skeletal muscle microvasculature stimulates increased capillary blood flow and enhances glucose uptake (103), central nervous system administration of insulin does not increase capillary perfusion nor glucose uptake in muscle (104). These data would indicate that insulin's action to increase capillary blood flow within muscle is a localised response and mediated through direct effects on the microvasculature. In addition to this, the microvasculature is one of the first tissues to lose its response to insulin during the development of insulin resistance and this is closely associated with reduced insulin-mediated glucose disposal (105-108). Thus, while it has been well established that insulin acts to increase capillary perfusion, identifying exactly where and how insulin exerts its effects in the vascular tree has remained elusive. Importantly, studies have shown that insulin mediates its vascular actions through nitric oxide and endothelin-1 (109-118). Moreover, Spiranac et al. recently reported that endothelin-1 provokes strong and stable contraction of individual capillary pericytes in mouse cremaster muscle in vivo via a Ca^{2+}-mediated mechanism (13). Coupled with the knowledge that muscle capillaries are almost completely covered by pericytes (figure 1C), that insulin can act directly on pericytes in other organs (30), and that pericytes are lost from skeletal muscle capillaries in type 2 diabetes (119), it is tempting to speculate that pericytes may contribute to insulin's actions on the muscle microvasculature and that these may be lost in insulin resistance. Whether this hypothesis is correct is currently not known and warrants future investigation.

Conclusions and future directions

In this review, we have highlighted the emerging evidence for pericyte-mediated regulation of capillary blood flow across various organs within the body. While the concept that pericytes actively regulate capillary blood flow in muscle is in its relative infancy, current studies suggest that (i) pericytes are present around skeletal muscle capillaries (figure 1; (13, 14)); (ii) skeletal muscle pericytes have the capacity to regulate capillary diameter (13, 14) and (iii) degeneration of capillary

pericytes occurs in skeletal muscle during type 2 diabetes (119). Based on the emerging repertoire of pericyte functions in other organs, the immediate need in skeletal muscle research is to (i) determine whether skeletal muscle pericytes are able to actively regulate capillary tone in response to circulating and metabolic factors; (ii) determine how pericyte loss from the microvasculature impacts muscle function and microvascular responses; and (iii) characterise the anatomical locations of pericytes in skeletal muscle and determine whether this differs between different muscle fibre types. Based on what is known about pericyte function in other organs and the emerging evidence in muscle, we hypothesize that pericytes as act as gatekeepers, controlling muscle nutrient exchange, coupling metabolism to microvascular supply (see figure 2 for working hypothesis). Given the strategic location of pericytes between capillaries and muscle fibres, unravelling their functions in skeletal muscle will significantly advance our understanding of muscle physiology during health, exercise and in disease states such as insulin resistance and exercise intolerance.

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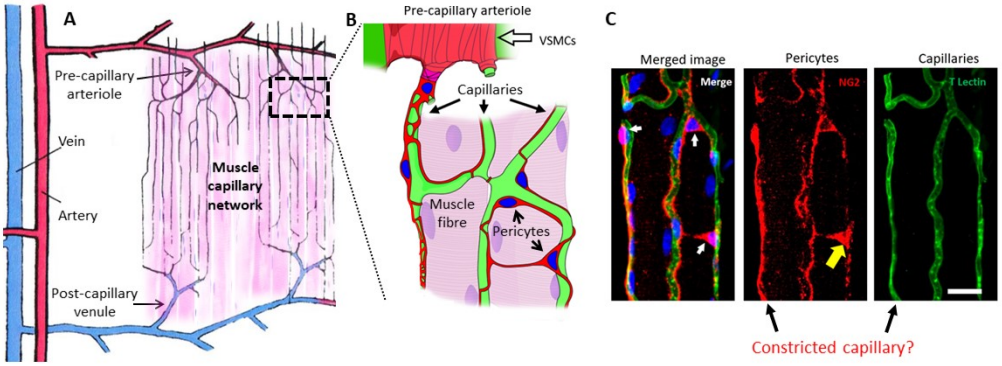
Figure legends

Figure 1. Skeletal muscle capillaries are covered by pericytes. Diagram depicting the structure of the microvasculature in skeletal muscle (**A**) highlighting the transition from pre-capillary arterioles covered by vascular smooth muscle cells (VSMCs) to capillaries covered almost completely by pericytes (**B** and **C**). In panel **C**, we performed immunohistochemistry on longitudinal sections of rat tibialis anterior muscle to assess pericyte (red; anti-NG2 antibody; Merck Millipore Cat.No. AB5320) coverage of capillaries (green; tomato lectin; Vector Labs; Cat.No. DL-1174). Nuclei were stained with DAPI (4',6-diamidino-2-phenylindole) and are shown in blue. White arrows point to pericyte cell bodies; the yellow arrow highlights a pericyte process extending across a muscle fibre to wrap around two adjacent capillaries. Scale bar = 25µm.

Figure 2. Working hypothesis where pericytes dilate capillaries to regulate blood flow and skeletal muscle metabolism. 1. Circulating factors such as insulin (I; shown in green) act on endothelial cells to simulate nitric oxide (NO) production by endothelial nitric oxide synthase (eNOS). NO diffuses from endothelial cells and stimulates pericyte relaxation. **2.** Insulin transitions from the capillary lumen into the interstitial space through either transcellular or paracellular pathways and directly acts on pericytes to stimulate potassium (K^+)-dependent hyperpolarization and relaxation. **3.** Muscle metabolism derived molecules, including carbon dioxide (CO_2), lactate (Lac) and adenosine (Ad), diffuse from muscle cells and act on pericytes to cause relaxation. **4.** In each case above, pericyte relaxation causes an increase in capillary diameter, enhancing delivery of glucose for storage as glycogen in the presence of insulin or to facilitate muscle metabolism.



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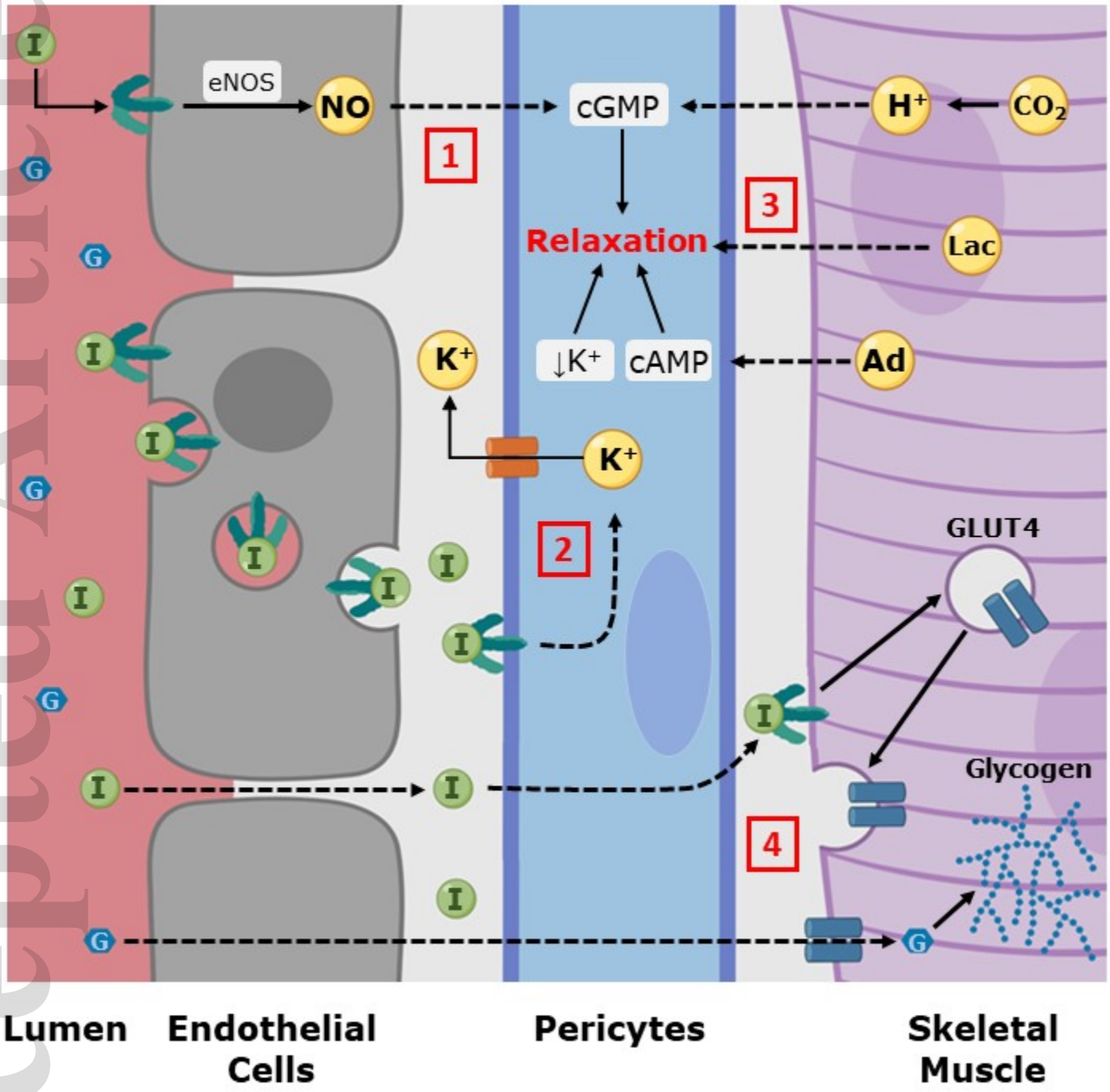

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